Standard Operating Procedures for Animal Experimentation (SOPs)

Prepared initially by ILRAD's Institute Animal Care and Use Committee (IACUC) on 2-8-94 and revised 29.12.94

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Contents

Introduction

This manual contains the Standard Operating Procedures (SOPs) for animal experimentation at ILRI. The SOPs are for those invasive procedures, which are routinely used within the institute. The number of the SOP should be used on the Experimental Animal Request Form and the procedure **AS WRITTEN** followed during the experiment. Any procedure not listed within this manual must be considered as a NON SOP and be described in full on the animal request form.

Suitably qualified personnel who use the procedures frequently wrote each SOP. Members of the Institute Animal Care and Use Committee and other frequent users then reviewed the SOP. Particular attention was paid to international guidelines for animal care and use in order to minimise discomfort to the experimental animal and to the ease of following and repeating the procedure. Following review and discussion modifications were made if considered necessary.

SOPs are prepared and used for several reasons related both to good science and to proper animal care. The main ones are a) that they reduce sampling error by utilising some level of consistency in data collection, b) they attempt to cause minimal stress to the animal.

We have not listed the cadre of personnel for each of the SOPs as we do not believe that for the majority of procedures competence is measured by position. Appropriate qualified personnel will assess the suitability of each individual.

We cannot over-emphasis the value of demonstration and learning to gain proficiency in the techniques employed. Nor should we forget that the SOP does not end with either preparation or sample collection – watchfulness and care of all experimental animals is a continual priority.

Duration of experiments is dictated by protocol but those involving animals may be curtailed at any time at the discretion of the veterinarian in charge.

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1. Antibody Production

a) Monoclonal antibody fusion procedure in mice

Back ground: The potential of a protein to induce antibodies (immunogenicity) in mice depends on its non-relatedness with mouse proteins. In general, the more distantly related the species from which the protein was isolated, the less antigen will be needed to induce a good antibody response. Quantities per infection usually range from 1mg to 1g, although antibodies can be obtained with less material.

Species: Mice

Strains: BALB/cOlaHsd are used for the primary immunization.

Procedure: Immunisation procedure: Antigen in 0.5 ml of saline or Dulbeccos PBS is mixed with 0.5 ml of Complete Freunds Adjuvant (FCA) until a stable emulsion is formed. This is then injected aseptically intra-peritoneally into a BALB/c mouse.

After 3 weeks a drop of tail blood is collected, the serum harvested and tested for reactivity with the antigen. If reactivity is low a boost can be given with antigen in incomplete Freunds adjuvant (IFA).

When serum shows a high antibody titre to the antigen a large quantity of protein (more than 1 mg up to a maximum 2 mg) is injected aseptically (preferably intravenously) without adjuvant. The mouse is euthanised three to four days later and the spleen cells isolated for fusion.

b) Polyclonal production

Species: Rabbits

Procedure: Commercially available adjuvants that have no (or extremely limited) adverse reactions should be used. Use of Freunds Complete Adjuvant (FCA) stopped in April 2001. Only if there is published or demonstrable proof that viable alternatives are not available may FCA be used as an initial immunisation and thereafter Freunds Incomplete Adjuvant (FIA) must be used. Neither FCA nor IFA must be given by the intravenous route. The final dose using FCA or IFA must not be more than 50% adjuvant mixed with the antigen. There are two factors which affect the severity of reaction when FCA is used, site and volume. Rabbits may be inoculated; intradermally, subcutaneously, or intramuscularly. The maximum number of sites and volume per site allowed to be administered are as follows, Intradermally, 4 sites, volume 0.1 ml per site; subcutaneously, 4 sites, dose per site 0.25 ml; intramuscular 2 sites, 0.25 ml per site. Intramuscular is particularly painful in rabbits and should not be used if alternative sites are available. Strict adherence to sterile injection techniques must be adopted at all times. The hair must be clipped and the site swabbed with 70% alcohol prior to inoculation to reduce the risk of bacterial contamination.

Equipment: Sterile syringes and 21 - 25 gauge hypodermic needles. A new needle to be used for each animal.

Blood collection: **B**lood will be collected post-immunisation. The relevant SOP2 should be referred to dependant on species.

2. Blood Collection

a) Blood smears

Species: Cattle, Goats, Sheep, Pigs, Rabbits.

Restraint: Crush or manual restraint.

Procedure: An ear vein is punctured with a fine (22 G) sterile needle. A drop of blood is allowed to fall on a slide and a smear made. Use a sterile swab to swab gently or apply pressure if bleeding continues.

b) Cardiac puncture

Species: Guinea Pigs, rabbits, rodents

Restraint: Animal is anaesthetised and restrained by either hand or tied down.

Procedure: This is only permitted on anaesthetised animals. The animal is placed in dorsal recumbency, the thoracic area swabbed with 70% alcohol and the area palpated to locate the area of the strongest heart-beat, in the region of the 5th and 6th ribs. A 25–27 gauge needle for rodents and Guinea pigs, 21–23 gauge for rabbits is then inserted at an angle of approximately 45 degrees through the intercostal space just to the left of the animals sternum into the heart and the blood withdrawn. Maximum blood volume allowed to be taken: mice 0.75 ml, Rats 5 ml. rabbits 30 ml.

c) Ear vein

Species: Pigs and goats

Restraint: Small pigs and goats can be held. Large pigs are restrained using a snare on the upper jaw behind the incisors.

Preparation: The outer surface of the ear is swabbed with 70% alcohol.

Equipment: 20g needle on a 5 ml syringe.

Procedure: There are several veins on the outer surface of the ear. Choose a large one and occlude it by digital pressure downstream from the collection site**.** Insert the needle into the vein at a shallow angle, in line with the vein, and gently withdraw the plunger of the syringe. If the vein collapses, release the pressure on the plunger until the vein refills, then try again. 5 ml is the maximum amount that may be collected.

Species: Rabbit

Preparation: The marginal vein or central ear artery are the chosen sites depending upon quantity required. Less than 2 mls can easily be collected from the marginal ear vein, more than this and less then 30 mls. can be collected from the central ear artery with a total maximum in 3 months of 50ml/kg. The hair is first plucked from the site of withdrawal. The ear is then rubbed vigorously to dilate the vessels and the site is swabbed with 70% alcohol ensuring that the surrounding hair is wetted. The vessel is occluded above the site of withdrawal by finger pressure.

Procedure: A 21-gauge needle is inserted into the vessel almost parallel to the vessels surface. With marginal vein collection a small nick may be made in the vein with a sterile blade and the blood collected directly from the site. When collecting from the central artery vacutainers may be used. Observe the blood flow and collect the required volume. On completion withdraw the needle and apply a wad of cotton wool with adequate pressure to the site until bleeding stops. Care must be taken when taking blood from the central artery to prevent haematomas by ensuring that the pressure is retained on the pad upstream from the puncture site after the bleeding for a sufficient period of time. The animal can then be returned to it's cage.

d) Jugular vein

Species: Cattle, Goat, Sheep, Eland, Buffalo, Waterbuck.

Restraint: Cattle over 100kg are held in a crush. The species head is turned 90 degrees from the line of the body and the nose tilted upwards by 30 degrees. Strong-necked cattle may need to be tied in this position with a rope halter ensuring that a quick release knot is used.

In smaller calves, goats and sheep restraint is most easily achieved by backing the animal into a corner, standing astride the shoulders and positioning the head in a similar manner to above with both hands.

All waterbuck, eland and buffalo, whether considered 'tame' or not, are sedated before collection of blood.

Preparation: The site is cleaned. In wool sheep the wool around the site may have to be clipped or plucked to ease visualisation of the vein.

Equipment: Either i) a sterile, unused vacutainer needle (18−20G), a plastic needle holder and an evacuated blood collection tube, or ii) a sterile, unused, Luer fitting needle (14−18G) with syringe attached.

Procedure: The jugular vein is raised by pressing the thumb or fingers into the jugular furrow at the base of the neck. With the head in the correct position, the jugular vein should be seen filling, running towards the angle of the jaw. In adult cattle it can be 3 cm in diameter, in small calves and adult goats and sheep it is around 1 cm in diameter. If in doubt tap the site with a finger; a wave of blood should be seen when the distended vein is tapped. If the vein still cannot be visualised, try to reposition the head.

The needle is pushed sharply through the skin and the wall of the vein at an angle of 30 degrees, in line with the vein. The vein is just under the skin so the needle should not penetrate more than 2 cm. When the evacuated tube is pushed onto the vacutainer needle or the plunger of the syringe is withdrawn, blood should be seen filling the tube or syringe. If there is no blood flow the needle is repositioned to puncture the vein without coming out of the skin.

Prior to removal of the needle pressure on the jugular furrow is withdrawn

Volume: The minimum amount of blood that is required should be taken. Excessive amounts of blood taken frequently can affect the P.C.V. particularly in smaller animals. The maximum volume of blood that should be taken from any animal is 5 ml/kg body wt. No more than this may be taken in any three month period. Animals that do have this maximum amount removed must be in good health and body condition.

e) Tail vein (*median coccygeal*)

Species: Cattle, buffalo, eland

This method is useful for mature bulls or where handling facilities do not allow easy access to the neck. It can only be used for animals greater than 250 kgs. and for blood samples less than 10 ml.

Restraint: The animal must be held in a race or crush with a bar behind to stop it reversing. ALWAYS BE AWARE WHEN STANDING BEHIND A COW THAT IT CAN KICK AT ANY TIME.

Equipment: A sterile, unused vacutainer needle (18–20G) a plastic needle holder and an evacuated blood collection tube.

Preparation: The tail is raised and the underside cleaned with 70% alcohol.

Procedure: The vein runs midline, just under the skin in a medial groove on the underside of the tail. The needle is pushed through the skin over this groove at a 90-degree angle. When the needle is felt touching the bone of the coccygeal vertebrae, it is withdrawn slightly. The needle will not penetrate more than 1 cm. When the needle is in position push the evacuated tube onto the needle. Blood should flow, if not, the needle should be repositioned without withdrawal from the skin.

f) Tail bleeding

Species: Rodents

The animal to be bled is maintained in a warm environment until the vein is visibly dilated. The veins lie on either side of the tail. A small portion at the end of the tail is excised using either sterile scissors or a scalpel blade; the tail is gently stroked from the base towards the tip and the blood collected either by pipette or into a container. Approximately 0.2 ml and 1ml from a mouse and rat respectively, can be collected by this method. With experience it is possible to withdraw blood by a 1 ml syringe and a 27-gauge needle directly from the tail vein in a rat.

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3. Bone Marrow Collection (sternal biopsy)

Species: Cattle

General: Sedation of cattle and biopsies are done under the supervision of a veterinarian. Cattle destined for a surgical procedure in which there will be heavy sedation or anaesthesia and lateral casting must not have food or water for 18 hours prior to surgery to help prevent regurgitation and possible development of aspiration pneumonia. Experimental cattle are weighed prior to the procedure to allow drug dosage determination.

Restraint: The cattle are restrained in the crush by a head stanchion and then sedated by intravenous injection (via the jugular vein) of 0.05–0.2 mg/kg of xylazine (during trypanosome infection the dosage is administered at 0.05 mg/kg because the cattle become increasingly more susceptible to sedation both due to illness and anaemia). The animals are allowed to move into a holding pen and once they show signs of sedation they are cast in lateral recumbency. The site in which the animal is cast in lateral recumbency should not be bare concrete but should be covered with wood shavings to prevent trauma to the animals' musculoskeletal tissue.

N.B. if animals are severely anaemic the amount of time the animals are in lateral recumbency should be kept to a minimum to prevent hypoxia developing in organs and tissues (xylazine causes hypotension in sedated animals).

Procedure: The sternum is shaved with a razor or battery operated electrical shaver equipped with a surgical blade (no. 40). The chosen biopsy site is aseptically prepared by first cleaning the site with Savlon soap (diluted 1:30 in 70% ethanol) and gauze pads, followed by two swabs with gauze pads soaked in 70% ethanol. The periosteum, muscle, connective tissue and skin are infiltrated with 5–10 ml of 3% lignocaine hydrochloride depending on the size of the animal.

The person performing the biopsy must wear gloves and should keep all needles, surgical blades etc, sterile. A small incision approximately 0.5–1.0 cm in length is made through the skin with a sterile No 11 surgical blade. A sterile 11-gauge 4-inch Jamshidi-type biopsy needle is used to penetrate the marrow cavity of any of the third, fourth, fifth or sixth sternebrae. Bone marrow samples for cytology require the collection of .05 ml of bone marrow into a 10 ml syringe without anticoagulant prior to the collection for in vitro culture. Cytological smears are made immediately and rapidly air-dried. Approximately 3 ml of bone marrow is collected into a second 10 ml disposable syringe containing preservative-free heparin in Iscove's modified Dulbecco's medium (IMDM) at a final concentration of 100 IU/ml. The biopsy site is sprayed with an antibiotic solution (Alamycine). Core biopsies can be collected with the same needle but require the needle be partially removed from the penetration site and redirected in another area of the marrow. This prevents distortion of the marrow structure and haemorrhage into the core biopsy. A fresh site should be selected for repeat biopsy. This is fairly easy to determine because of a small pinkish scar on the skin from the previous week. The older scars will be white.

The animal is manually moved to the side of the pen and placed in sternal recumbency. The animals are kept in this pen until they fully recover from the sedative and are able to walk around the pen in a normal manner. They are then returned to their holding pen and allowed to eat and drink approximately 2 hours later.

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4. CSF Collection by Cannulation

A method for repeated sampling of lumbar cerebrospinal fluid in goats

Species: Goats

Background: The cerebrospinal pathway comprises the ventricles of the brain, the central canal of the spinal cord, the subarachnoid space and perivascular extensions of this space. Contained within this pathway is cerebrospinal fluid (CSF), which has the function of protecting, nourishing and maintaining the homeostasis of the brain and spinal cord. Single or repeated sampling of CSF can be achieved through a puncture made into the cerebral ventricles, or at one of two levels of the subarachnoid space; the *cisterna magna*, or in the lumbar region at a point beyond the termination of the spinal cord. In the absence of adequate restraint, or when a faulty technique is employed, the nervous system may be traumatised and also may result in contamination of CSF by blood cells.

The following technique has been developed for repeated sampling of cerebrospinal fluid from conscious goats by means of a catheter placed surgically in the subarachnoid space between the sixth and seventh lumbar vertebrae. Uncontaminated cerebrospinal fluid in excess of 1.0 ml can be obtained readily from the cannulated goats several times daily for up to 6 weeks.

Procedure: Cannulation apparatus: The epidural apparatus used is the Minipack which comprises of a 10.5 cm long 18 G Tuohy needle (outer diameter 1.3 mm, inner diameter 1.0 mm) with graduations at the 3, 5 and 7 cm levels. The lumen of the needle is guarded by a plastic probe. A detachable plastic wing for ease of handling of the needle during operation is also supplied. The catheter is 90 cm long, nylon (outer diameter 0.9 mm), and open only at one of its two ends. The closed end, marked with 3 lateral "eyes", is used for catheterisation of the subarachnoid space between the sixth and seventh lumbar vertebrae. The pack also includes a transparent tube for guiding the catheter into the needle, and a Luer lock connector consisting of upper and lower halves. The lumen of the lower half of the connector is guarded by an insert tube and a cover is included for closing the upper half of the connector.

Presurgical care: Goats are starved for 24 hours prior to beginning the surgical procedure. A rectangular area (40 cm × 15 cm) is then clipped and shaved in the lumbosacral region, with the animal in the standing position. The shaved area includes the areas immediately cranial and caudal to the median sacral crest and the tubera coxae. The longitudinal axis along the lumbosacral spine region is then marked with a straight interrupted line, using a marker pen. Subsequent to anaesthesia and placement of the animal in left lateral recumbency, the line aids in placement of the skin in its normal position in relation to the spinal longitudinal axis.

Routinely, each goat is given 0.2 ml of 2.0% xylazine intravenously to ease induction of anaesthesia. Following premedication, the goat is then placed in left lateral recumbency on the surgical table, then anaesthetised using a 5% solution of thiopentone sodium administered dose-to-effect via the right jugular vein. Immediately after induction of anaesthesia the goat is then intubated with a size 8 endotracheal tube. After connection to an anaesthetic machine, goats are maintained in surgical anaesthesia using an oxygen-methoxyfluorane-halothane mixture in a semi-closed system. The four legs of the animals are then tied together to ensure the spinal column is in a convex position. Thereafter, the shaved area of skin above the sixth and seventh lumbar vertebrae is washed with a 1% solution of Savlon, and cleaned with a cotton swab. Sterile drapes are then clipped to the trunk of the animal, leaving the surgical area exposed, and strict asepsis is maintained throughout the surgery.

Surgical procedure: Immediately prior to surgery, the epidural Minipack is unpacked and the different parts identified and assembled. A 5 mm long incision is then made into the skin overlying the subarachnoid space between the sixth and seventh lumbar vertebrae. The Tuohy needle, containing the lumen guard, is then inserted into the incision with the curved end orientated in a cranial direction. The

winged outer end of the needle is then used to slowly advance the needle in a ventral direction through the tissues. When the 3 cm mark on the needle is at the level of the skin incision, the probe guarding the lumen of the needle is withdrawn. Gradually, the needle is advanced deeper until access into the subarachnoid space is achieved. If anaesthesia is not sufficiently deep, entry into the space is accompanied by short-lasting opisthotonos. The appearance of clear normal CSF at the outer end of the needle indicates a successful cannulation. Immediately after appearance of the fluid, the catheter is inserted rapidly through the needle into the subarachnoid space. Care is taken to ensure that the correct end of the catheter is used for the catheterisation. After approximately 7 cm of the catheter has been introduced into the space, the needle is carefully withdrawn, leaving the catheter in place. A pair of scissors is then used to cut off the excess portion of the catheter so that approximately 8 cm remains outside the incision site. The outer tip of the catheter is then positioned in the connector assembly, to a level just above the stop washer in the connector's lumen. The upper and lower halves of the connector are then twisted securely, but not too tightly, to fasten the catheter. At this stage, a sterile 1.0 ml syringe is used to obtain a reference CSF sample. The white screw-cover is then used to close the outer end of the upper half of the connector.

In order that the catheter is not accidentally pulled or damaged by the goat when conscious, a rectangular sheet of elastoplast measuring approximately 8 × 6 cm, is placed on the shaved skin, immediately cranial to the cannula insertion site. The connector is then placed on the elastoplast in a cranial orientation, and another, but shorter (6 × 4 cm), piece of elastoplast used to affix the connector to the underlying elastoplast. The two sheets of elastoplast are then jointly sutured to the skin area using approximately six silk 2O sutures. In this way, the connector is fixed semi-rigidly in position.

For each goat, the total time taken for the entire cannulation procedure usually varies between 30 and 60 min. At completion of the procedure, each goat is treated intramuscularly with oxytetracycline at a dose of 20 mg/kg body weight. An oxytetracycline spray is also applied to the incision area. Generally, following some degree of ataxia, goats are able to stand and resume feeding within one hour of the operation. Thereafter, the animals can be housed communally. Goats do not interfere with each other's catheter assembly, presumably because the associated irritation causes animals to move away from each other.

Sampling procedure for collection of CSF: Sampling is carried out with the aid of an assistant restraining the goat in a standing position. The operator's hands are cleaned with a solution of 70% alcohol, before removal of the connector cover. Using a sterile 1.0 ml syringe tightly fitted to the mouth of the connector, the desired volume of CSF is then gradually aspirated. When the flow of CSF is compromised, infusion of 0.2 ml of 200 iu/ml of heparin solution in sterile distilled water at 27 C into the catheter is usually found to restore the flow. At completion of the sampling, the connector cover is returned into position, but without over tightening.

At the termination of an experiment, goats are given 0.2 ml of 2.0% xylazine i.v. 3.5 min prior to the removal of the cannula. With the goat restrained in a standing position, the cannula is then removed from the intervertebral space by a single rapid movement.

Reference: Peregrine, A.S. and Mamman, M. 1994 A simple method for repeated sampling of lumber cerebrospinal fluid in goats. *Laboratory Animals* 28:361–369.

Other animal species: Although the above technique has not been applied to other animal species, there is no reason why it should not work. In adult cattle a 16 gauge cannulation apparatus should probably be used.

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5. Cell Depletions

Species: Mice, cattle

Background: Lymphocytes can be depleted *in vivo* by injecting an anti-lymphocyte antiserum. It is generally assumed the lymphocytes are coated by the antibodies and lysed by complement. This technique was first developed in man for prevention of tissue and organ rejection. When more specific monoclonal antibodies were developed in the late seventies, it became possible to deplete specific lymphocyte subpopulations. This method has less side effects and leaves other parts of the immune response intact. Therefore, depletion of specific cell subpopulations is a clean and less interfering way of dissecting an immune response in vivo. So far, successful lymphocyte depletion's have been performed in mouse, rat, man, monkey, cattle and sheep.

Mice: All depletion's in mice have been done by single or multiple intraperitoneal injections. The antibodies are given as purified antibody or ascitis. No adverse reactions have been mentioned, except the effect that the absence of a particular immune population may have on the disease studied (which may be beneficial). Amounts of antibody used range from 200 micrograms to milligram quantities.

Ruminants: Depletion's in cattle and sheep are done by intravenous injection of a specific mouse monoclonal antibody. Subcutaneous administration did not work well.

Side effects: Side effects have been reported with administration of antibodies by drip in human patients. They varied from sweating to slight temperature rises 3 hours after administration. In monkeys severe side effects (including shock-like symptoms, vomiting and even death) were observed, especially with CD8 antibodies. In preliminary studies with cattle such severe reactions were not observed. The following symptoms were seen within one minute after intravenously injecting too high quantities: fast breathing, problems with equilibrium and in the worst case dropping to the floor and rolling of the eyes. Except for the fast breathing, which could last up to 20 minutes, these symptoms lasted from a few seconds to a couple of minutes. It was found that these symptoms were due to presence of cells carrying the T cell antigen in the blood. The severity of the side effects further depended on the total amount of antibody given, but not on the antibody concentration in the syringe or the speed of injection. As soon as positive cells are eliminated from the blood no adverse effects develop, and up to 20 ml of ascitis have been given without problems. It is hypothesised that the symptoms are due to temporary agglutination of cells in the blood, or maybe by release of biochemical mediators from the lysed cells.

The following procedure, in which the dose of antibody is gradually increased, can be used to deplete T cells. As soon as one of the adverse reactions described above is observed, the next dose 1−2 hours later must not be increased. Only if no adverse reactions are observed can the dose be increased.

Procedure: Ascitis containing 5−10 mg IgG/ml is filter sterilised through 0.45 micron filters.

The following schedule will eliminate all CD4 or CD8 T cells from the body of an animal of 50−150 kg, without side effects.

Day 1: 4 I.V. injections of 25 microliter ascitis, with 1−2 hour intervals.

Day 2: 4 I.V. injections of 25, 50, 200 and 500 microliter respectively, with 1−2 hour intervals.

Day 3: 4 I.V. injections of 100 ul, 500 ul, 5 ml and 20 ml respectively, with 1−2 hour intervals.

Reappearance of cells of the depleted phenotype can be measured in the blood and should take at least two weeks. More antibody can be administered using the same schedule, if depletion is needed for longer times, but anti-mouse-Ig is present by day 5 and may weaken the effect of additional injections of monoclonal antibody.

When the experiment is over, one must consider that the animals may have lost part of their immune system and precautions taken to prevent infection.

6. Euthanasia

The term "euthanasia" is used to describe the process whereby an animal is killed using a recognised and acceptable humane technique. By derivation it means "good death" and thus carries the explicit implication of a quiet, painless death without fear or anxiety. The most important criterion of acceptance of a euthanasia method as humane is that it has an initial depressive action on the central nervous system (CNS) to ensure immediate insensitivity to pain.

It is important to recognise that some methods of euthanasia which cannot be made aesthetically pleasant, such as decapitation or stunning with exsanguination, may nonetheless be humane in terms of the above criterion. This concept is important to keep in mind when deciding on the method of euthanasia to be used. The choice must be based on the sensibilities of the animal to be killed rather than the sensitivities of the observer or operator, although the latter should not be disregarded.

In humane killing the person doing the job is the most important factor.

The method chosen will depend upon the nature of the study, the species of animal, and the number of animals to be killed. In some cases it may be necessary to handle each animal individually; in others, several animals or groups may have to be handled simultaneously (mass euthanasia). Regardless of whether individual or mass euthanasia is undertaken, the procedure followed must always attempt to meet the following criteria:

a) Death without signs of panic, pain or distress.
b) Minimum time to loss of consciousness or shortest lag phase.
c) Reliable and reproducible.
d) Safety to personnel involved.
e) Minimal undesirable physiological and psychological effects to tissues.
f) Compatibility with the requirement and purpose of the study.
g) Minimal emotional effects on the observer and operator.
h) Minimal environmental impact through contamination.
i) Simple, inexpensive, relatively maintenance free mechanical equipment.
j) Location remote and separate from animal rooms.

Methods for Euthanasia

The most important consideration in the choice of methods for euthanasia is the proficiency of the operator. At ILRI the following methods are approved.

a) Chemical

Species: All

Carbon dioxide: This may be used for all common laboratory animal species employed within the Institute, the governing factor is the volume required. Animals are placed in an airtight container with ventilation port, a specifically designed CO2 chamber or a plastic bag in which either a small group of animals or a cage of animals has been placed. In the case of a bag most of the air should be dispelled. The CO_2 is then slowly introduced and the animals will drift into unconsciousness. The chamber must not be filled with CO_2 before the animals are placed within, this will cause distress due to the lack of oxygen. The animals should be retained in this environment until rigor mortis has set in, or at least until all signs of life have ceased. If the animal is to be exsanguinated it is removed from the CO2 chamber once consciousness has been lost and all the blood quickly removed so that the animal does not regain consciousness. No animal should be disposed of until "rigor mortis" has set in.

Overdose of Euthatal: Pentobarbitone Sodium B.P. (200mg/ml): This must be administered quickly by the I.V. route. Care must be taken that the animal to be euthanised is well restrained so that the total dose may be given quietly and effectively. Dosage 1ml Euthatal to 1.4 Kg. body weight.

NB for cattle, A saturated solution of magnesium sulphate may be used in conjunction with barbiturates by the I.V. route. The animal should first be anaesthetised by a barbiturate and then the saturated solution of magnesium sulphate solution given until the heart stops.

b) Exsanguination

Species: Rats, mice, guinea pigs and rabbits

Procedure: The animal is first given an intravenous injection of phenobarbitone, thiopentone or CO2 inhalation to render it under full anaesthesia.

Once the animal is under full anaesthesia it is placed in dorsal recumbancy and restrained. The area where the strongest heart-beat can be felt through the rib cage is swabbed with 70% alcohol. An 18–25g needle is then inserted horizontally through the intercostal space into the heart. Blood is withdrawn until the animal is dead, care being taken to ensure that the heart beat has stopped and that rigor mortis has set in prior to disposal of the cadaver.

c) Exsanguination by carotid artery cannulation

Justification: To exanguinate an animal and collect the blood.

Species: Cattle

Site: Jugular furrow

Restraint: General anaesthetic with thiopentone to effect.

Preparation: After induction with thiopentone the animal should be made to fall on it's right side.

Equipment: A surgical blade and holder plus a cannulation needle, the diameter of which should be at least half the diameter of the artery.

Procedure: A 15 cm incision is made along the jugular furrow, dorsal and parallel to the jugular vein. The muscles dorsal to the jugular vein are cut along their fibres exposing the fascia below. The carotid artery is located by digging the finger into this fascia and locating the pulse. The artery is grasped and exposed, freeing surrounding fascia and vagus nerve. A small, shallow cut is made in the arterial wall and the cannulation needle pushed through into the lumen of the artery. When the stylet is removed from the needle the blood flows. The animal must not be allowed to recover.

d) Physical

I) Captive Bolt

Species: Cattle, sheep and goats

A captive bolt pistol may be used for pre stunning these animals whether for euthanasia or where the meat may be salvaged after exsanguination. The use of the captive bolt pistol must be undertaken only

by trained licensed experts. Following shooting with this instrument, the major blood vessels and spinal column should be severed and the animal exsanguinated.

ii) Cervical dislocation

Species: Mice and young rats

The mouse or young rat is placed on a non-slip surface; the base of the tail is gripped firmly. A rod, such as a ballpoint pen/pencil or the fingers is used to firmly hold the animal behind the base of the skull. The rod or fingers are then pressed down while the tail is raised about 30–40 degrees from the horizontal and a firm pull exerted away from the body. The dislocation can be felt through the hands of the operator.

Species: Rabbits

The hind legs are gripped firmly in one hand; the other hand is placed from above the rabbit with two fingers around the rabbit's neck and under the jaw. The rabbit is then stretched vigorously and the head jerked backwards at the same time. This should not be attempted on rabbits in excess of 3 kgs. weight. The dislocation of the spinal column will be felt.

Species: Guinea Pigs

The Guinea pig is placed on a smooth surface; the fingers from above the animal are placed around the neck and under the jaw. The animal is then jerked off the table and sung downwards vigorously, the swing is stopped abruptly. The weight of the body and the cessation of the swing will cause the dislocation. The operator will feel the dislocation.

iii) Decapitation

Species: Mice, rats and Guinea pigs and small rabbits.

A guillotine may be used for Guinea pigs, rats and mice, and rabbits of less than 1 kg. body weight.

Newborn mice may be killed by quickly cutting off their heads with sharp scissors.

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7. Injections

General Procedure: For all injections an unused, sterile needle must be used. The gauge of the needle is determined by the volume of material to be injected, the viscosity of the material, the site and the size of the animal to be injected. Always use the smallest gauge needle practicable. (see attached tables)

The skin must be clean and aseptic techniques must be adhered to.

Ensure the animal is well restrained prior to injecting. A sharp confident injection is easier on the animal than a weak indecisive one. Syringe and needle must be held steady to reduce pain and destruction of tissue.

a) Intradermal

Species: all species

Site: Loose skin on neck or flank

Procedure: A wide bevelled needle is used. The material to be injected is deposited in the dermis, which may be 1 cm thick in adult cattle but only 1 mm in mice. The needle is inserted so that the point just goes into the skin but does not penetrate the subcutaneous tissue below. As the material is injected a bleb can be felt in the skin. No more than 0.1 ml can be injected.

b) Intramuscular

Species: All species

Site: For animals over 70kg use the gluteal muscles on the rump or the trapezius muscle in the neck. The neck muscle should be the muscle of choice in cattle, sheep and goats that are subsequently going for meat. For animals under 70kg use the *vastus lateralis* muscle in front of the femur.

For small laboratory animals any muscle of suitable size can be used.

Procedure: The needle is pushed through the skin at right angles and into the body of the muscle. Withdraw plunger of syringe before injecting to ensure a blood vessel has not been penetrated. Then inject substance slowly.

c) Intraperitoneal

Species: Small laboratory animals

Site: With the animal restrained firmly in one hand with the head tilted downwards so that the abdominal contents are towards the back and away from the injection site (or in someone else's hands, depending on the size of the animal).

Procedure: A 2–3 cm. 27–23 gauge needle is used. The needle is inserted in the right inguinal region in a cranial direction first through the skin and then through the musculature into the abdominal cavity. The plunger is then sucked back to ensure no vessels have been penetrated.

Species: Lambs and Goat kids

Site: Ventral abdomen, midline, just caudal to the xiphoid cartilage. The animal should be held vertically so that the abdominal contents sink.

Procedure: The needle is pushed through the skin and muscle and can be felt entering the abdomen. Withdraw plunger of syringe before injecting to ensure no abdominal organ (liver, stomach) is penetrated.

d) Intravenous

Species: Cattle, Sheep, Goats and pigs

Site: Jugular

Procedure: The jugular vein is raised by pressing the thumb or fingers into the jugular furrow at the base of the neck. With the head turned 90 degrees and held slightly upwards, the jugular vein should be seen filling, running towards the angle of the jaw. In adult cattle it can be 3 cm in diameter, in small calves and adult goats and sheep it is around 1 cm in diameter. If in doubt tap the site with a finger; a wave of blood should be seen when the distended vein is tapped. If the vein still cannot be visualised try to reposition the head.

The needle should be pushed sharply through the skin and the wall of the vein at an angle of 30 degrees, in line with the vein. The vein is just under the skin so the needle should not penetrate more than 2 cm. Blood is withdrawn to ensure the needle is in the vein, then the material is injected slowly into the vein.

Species: Rabbits

Site: Marginal ear vein

Procedure: The rabbit is restrained either in a restraining box or wrapped in a towel or lab coat. The hair on the ear is plucked in the region to be injected. The ear is massaged to dilate the vessel. Site is swabbed with 70% alcohol and the vessel occluded below the injection site (nearer the rabbit's head), this will cause dilation of the vein. The needle (maximum size 23 × 2.5 cm.) is inserted into the vein almost parallel to the vessel surface. The plunder is withdrawn until a small volume of blood is seen in the syringe. The occluding pressure is then released. The material is then injected slowly into the vein. If an increase in resistance is felt on the plunger, observe to ensure that the needle is still well positioned within the vein. On completion the site is gently squeezed and the needle withdrawn.

Species: Rodents

Site: Tail veins

Procedure: The animals must be well restrained either manually or by a restraining device. The tail is warmed until the veins are clearly visible on each side of the tail. The base of the tail is occluded to dilate the vessel. The tail is swabbed with 70% alcohol; a 27g × 1 cm needle is inserted almost parallel and into the vein. Once within the vein the occluding pressure is released. It is not always possible to withdraw a small quantity of blood. When the needle is placed properly in the vein it is comparatively easy to slowly depress the plunger, and the material can be observed flowing through the vein. Any slight swelling or increased resistance indicates that the needle is not positioned properly. The other vein or if possible a site above the original attempt must be used. The plunger must be depressed slowly.

e) Subcutaneous

Site: Loose skin at the back of the neck

Procedure: The skin is pinched and the needle pushed through the skin at right angles so that the point is in the subcutaneous fascia below the skin. Withdraw plunger of syringe before injecting to ensure a blood vessel has not been penetrated.

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8. Lymph

a) Duct cannulation

Species: Cattle

The most convenient duct for cannulation in cattle is the efferent prescapular. Afferent lymph can be collected from this after removal of the node; a period of approximately 8 weeks is required for re-anastomosis.

Procedure: The animal is starved for at least 24 hrs and water with held for 12 hours prior to surgery. The animal is anaesthetised by sedation with xylazine (0.2 mg/kg), entubated, administered Halothane, and placed in lateral recumbency.

The duct runs from the node to the jugular vein (R) or thoracic duct (L) along the medial surface of the *brachiocephalicus* muscle. It is generally associated with the prescapular artery and vein, although this association may not be close. The duct is approached via a skin incision along the jugular groove of approximately 10 cm that is roughly bisected by a perpendicular from the lymph node. The subcutaneous fascia and the connective tissue that joins the *brachiocephalicus* and *sternocephalicus* muscles are blunt dissected and the *brachiocephalicus* muscle is reflected upwards and held in position with retractors. The prescapular artery and vein should be visible deep in the cleft between the two muscles. The duct is usually cranial to the vessels, and traverses the gap between the muscles along with them. It varies in size and usually appears as a rather translucent ribbon-like structure that can be mistaken for a nerve. Injecting a solution of Evans blue dye in the drainage area of the node can help in identifying it.

It is best to cannulate the duct where it is associated with the inner surface of the *brachiocephalicus* muscle. It is closely associated with fascia and this stabilises the cannula and lessens the possibility of a kink.

Polyvinyl chloride cannula (Dural Plastics, Australia) are most suitable and for *Bos indicus* cattle it is best to use dual lumen tubing so that an anti-coagulant can be pumped up to the tip. Single lumen tubing may suffice in *Bos taurus*. Cannulas can be sterilised with ethylene oxide or immersed in chlorhexidine. The duct is tied off with 4–0 silk, distal to the point selected for insertion of the cannula. The swollen vessel is carefully cleaned of fascia for approximately 0.5 cm proximal to the tie (use fine forceps), and another tie is laid at this point. The cannula is now introduced to the site and led through the *brachiocephalicus* muscle to emerge at the point of the shoulder using a long (15–20 cm is enough) stainless steel needle with a hole at the blunt end through which the cannula will pass. The cannula is now filled with Alsever's solution and clamped at the distal end. To insert the cannula in the duct, the vessel is cut between the two ties (about half of its circumference) with corneal scissors. The cut edge of the duct may be picked up with very fine forceps to aid in the insertion of the cannula. It is desirable to have at least 1.5 cm of cannula in the duct. In many cases the ductal valves hinder this, but these can be broken with a lachrymal duct probe. Once the cannula is in place, the second tie is secured. Lymph should now flow.

The surgical site is closed in two layers using catgut for the connective tissue between the *brachiocephalucus* and *sternocephalicus*, and Supramid for the skin. It is useful to put some adhesive tape around the cannula where it emerges from the skin and suture this to the skin. Allow about 0.25 cm of cannula between the tape and the skin.

If using a dual lumen cannula, the calf must be restrained by neck bales so that a peristaltic pump can be used to pump Alsever's solution up the cannula. If not, a plastic bottle can be sutured to the skin to collect lymph. Heparin/pen/strep powder is added to the bottle so that the lymph doesn't clot.

b) Lymph node biopsy (smears)

Species: Cattle, sheep, goat

Site: Parotid, pre-scapular or pre-femoral lymph node

Procedure: The site must be clean and aseptic technique adhered to. The lymph node is immobilised between thumb and forefinger of one hand and a 20G needle is pushed into the centre of the lymph node with the other hand. The plunger of the syringe is withdrawn until a small volume of lymph is seen in the hub of the needle. The needle is removed and the sample is deposited on a microscope slide and a smear is made. If the sample is frank blood, repeat the procedure with a new needle.

c) Lymph node removal (partial and complete)

Species: Cattle

Site: Pre-scapular or pre-femoral

Restraint: General anaesthetic with thiopentone and halothane. The pre-femoral lymph node can be removed under deep sedation and local anaesthesia

Procedure: The animal is starved for at least 24 hours and water withheld for 12 hours prior to surgery. Thiopentone sodium solution is administered intravenously at a dose rate of 1 g per 100 kg bodyweight. The animal is intubated and anaesthesia is maintained using gaseous anaesthetic. The area around the lymph node to be removed is clipped and scrubbed with a dilute savlon solution. Ethanol (70%) is then applied.

If sedation and local anaesthetic are being used, xylazine is given at 0.2 mg/kg and local anaesthetic is infused subcutaneously, in an L-block, anterior to the lymph node.

Strict aseptic technique must be adhered to throughout the operation.

Pre-femoral: a skin incision is made over the lymph node, which lies in subcutaneous fat. The fat is dissected away from the lymph node until the node is attached only at the hilus by the main artery and vein. These are clamped and cut and the node is removed. If a complete lymph node removal the artery and vein are ligated before removing the clamp.

Pre-scapular: This lymph node lies deeper, under a band of muscle. This muscle is cut prior to dissecting out the node, and sutured with absorbable sutures after removal of the node. The skin is sutured with interrupted, non-absorbable sutures.

Prophylactic: antibiotic (pen/strep) is then administered.

Post-operative care: skin sutures are removed after at least 10 days.

9a. Biopsy - skin

Species: Cattle, sheep, goat

Site: Flank or neck

Restraint: General anaesthetic with thiopentone 1gm/100 kg.

Preparation: The animal must be starved for at least 24 hours and water withheld for 12 hours prior to surgery. The site to be incised is clipped and scrubbed with a dilute savlon solution. 70% ethanol is then applied. Thiopentone sodium solution is administered intravenously at a dose rate of 1 g per 100 kg bodyweight. This should give around 10 minutes anaesthesia.

Procedure: Strict aseptic technique must be adhered to throughout the operation. An elliptical incision is made through the skin, around the site to be biopsied. The piece of skin is removed, only the minimum amount of skin required must be taken. The wound is closed using simple, interrupted, non-absorbable sutures. Prophylactic antibiotic (pen/strep) is administered.

Post-operative care: Skin sutures are removed after at least 10 days.

Site: A biopsy can be taken from the ear of cattle using an ear punch. The site must be cleaned with 70% ethanol prior to the punching. Care must be taken to avoid the major blood vessels in the ear. Topical antibiotic must be administered post-operatively.

Site: Flank or neck

Small biopsies can be taken using a 6 mm diameter, disposable, sterile, biopsy punch. (Stiefel Laboratories U.K.). The skin is clipped and scrubbed with savlon solution and the punch pushed through the skin. The biopsy remains in the punch when it is removed. Topical antibiotic is applied to the site.

9b Biopsy – Mammary gland

The site of biopsy is clipped and scrubbed with Savlon or equivalent. Local anaesthetic is administered. A 1mm biopsy needed is pushed through the skin to a depth of 5 cm removing 5 – 10 mg of tissue.

9c Biopsy – Adipose tissue

Adipose tissue may be taken from the mammary gland, the hump or near the tail head. The skin is shaved and clipped and a local anaesthetic applied as above. An incision of between 2 and 3 cm is made in the skin and then a small amount of the underlying adipose tissue is removed with a scalpel. The incision is sutured and treated with a topical antibiotic.

9d Serial Collection of Liver Biopsy from Cattle

A. Purpose:

The purpose of this procedure is to allow sampling of liver tissue from cattle in sufficient amount for biochemical assays at different stages during disease progression

following trypanosome infection. The procedure satisfies two key demands: serial liver tissues sampling from one individual while ensuring minimum distress to the animal. Overall the procedure allow to increase the power of the experiment by reducing the effect of individual variation of the sampling and so reducing the overall number of animals subjected to experimentation.

B. Scope:

Functional genomics experiments (ILRI). Sampling procedure to be performed by a skilled veterinarian.

C. Materials and Equipment:

Experimental cattle aged between 9 and 24 months.

Clean animal handling facilities (crush, roofed recovery pens, halters)

Medicines:
Sedative/analgesic e.g. Xylazine;
Local anaesthetic e.g. lidocaine
Antibiotic; Penicillin-Streptomycin

Surgical kit:
antiseptics e.g. savlon and 70%ethanol;
hair clippers,
19g hypodermic needles and syringes
scalpel blades and holders, scissors,
suture needles and holders, threads

Sterile 15 or 20 cm Vet-Core™ Biopsy needles

Nuclease free Cryotubes (biopsy receptacle)

Liquid nitrogen

D. Reagents and Equipment preparation

No special preparation of equipment is required, but utmost care is taken to keep the surgical site sterile by applying good veterinary practice. The procedure is performed in the morning before the subject is fed.

E. Procedure, step by step:

1. The animal must be held by the neck in a crush with easy access to its anterior flank on the right hand side.

2. Xylazine is administered at a dose rate of 0.05 mg/kg to achieve sedation while animal remains standing. Local anaesthetic is infiltrated around the site. The site is clipped and scrubbed with a 1 in 5 savlon solution and sterilised with a spray of 70% Ethanol.

3. The site is located at the intersection of the right 11^{th} intercostal space and a horizontal line extending cranially from the middle of the paralumbar fossa.

4. After the local anaesthetic has taken effect, a 2 – 4 cm incision is made in the skin and muscles in order to access to the peritoneal cavity. Care is used to avoid the intercoastal artery as it courses along the posterior aspect of the 11th rib.

5. The VetCore biopsy needle is passed through the incision to penetrate 2 – 3 cm into the liver and the biopsy is collected by firing the needle's trigger mechanism.

6. The needle is withdrawn, and the sleeve drawn to expose 15 – 20 mg of tissue that is quickly transferred into a labelled collection tube taking care not to take blood that may be on the needle. The collection tube with the specimen is immediately frozen n liquid nitrogen.

7. The skin incision is closed with 2 – 3 non absorbent sutures. The animals are covered with antibiotic and released into the holding pen from where they are monitored for 12 hours for any evidence of bleeding or shock.

§Adapted from Pearson, E.C.: Liver Disease in Equine and Food Animals. Modern Veterinary Practice. March, 1980, p. 230-237

F. Notes:

In place of the fine VetCore needles, custom built instruments such as the one shown in the picture below may be used. The latter have the advantage that only one successful entry will recover sufficient amount of tissue thus avoiding the need to penetrate the liver repeatedly.

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10. Spleen

a) Biopsy

Species: Cattle

Site: Intercostal space between the 11th and 12th rib, at the level of, the transverse processes of the lumbar vertebrae.

Restraint: The animal to be held by the neck in a crush with easy access to its anterior flank.

Preparation: Strict aseptic technique must be adhered to throughout the operation. The animal is starved for at least 12 hours prior to surgery. Xylazine is administered at a dose rate of 0.05 mg/kg (sedation but animal remains standing). Local anaesthetic is infiltrated around the site to be incised.

The site is clipped and scrubbed with a dilute savlon solution. Ethanol is applied to the site.

Equipment: A sterile Tru-cut Biopsy Needle, 10 or 15 cm. and a sterile surgical blade.

Procedure: A puncture incision is made in the skin prior to inserting the needle. The needle is kept horizontal and pushed through the intercostal muscles and the diaphragm. When the needle is in the diaphragm it moves with the animals breathing. At this position the point of the needle should be resting on the spleen. The needle is directed caudally and the stylet is pushed into the spleen. The sleeve is pushed over the notched stylet to cut the biopsy. The stylet is removed from the sleeve and the specimen taken from the notch. A repeat specimen can be taken as the needle is still in place. The needle is removed and the wound sprayed with antibiotic.

b) Spleen exteriorisation

Justification: To facilitate repetitive splenic biopsies.

Species: Cattle, less than 9 months old. These animals must not be crushed and must be handled with due respect for the exteriorised spleens.

Site: Laparotomy on left side via 12th rib.

Restraint: General anaesthetic.

Preparation: The animal is starved for at least 24 hours and water withheld for 12 hours prior to surgery. Thiopentone sodium solution is administered intravenously at a dose rate of 1 g per 100 kg bodyweight. The animal is assisted if necessary into lateral recumbency on the right side. The animal is intubated and anaesthesia is maintained using gaseous anaesthetic (halothane). The area around the 12th rib and the left paralumbar fossa is clipped and scrubbed with a dilute savlon solution. The site is swabbed with 70% ethanol.

Equipment: A sterile surgical kit plus sterile embryotomy wire with handles.

Procedure: Strict aseptic technique must be adhered to throughout the operation. A skin incision is made over the 12th rib from the costochondral junction dorsally for around 20 cm. Musculature overlying the rib is incised. The periosteum overlying the rib is also incised and the rib separated from the periosteum. Once the periosteum has been completely separated from around the rib, the rib is cut transversely using embryotomy wire around 12 cm from the costochondral junction. The body of the rib

is separated at the costochondral junction and the free piece removed. A 12 cm long incision is made through the periosteum and peritoneum into the abdomen.

The spleen is separated from the rumen and the diaphragm to allow an anti-clockwise rotation of 90 degrees around the hilus. The tail of the spleen can be exteriorised.

A skin pocket is made between the skin and muscle, posterior to the skin incision, by dissecting the subcutaneous fascia. This pocket must be made big enough to accommodate the exteriorised tail of spleen. The exteriorised tail of spleen is fixed in position using continuous, gut sutures on an atraumatic needle attaching the splenic capsule to the peritoneum and periosteum on both sides of the incision. The exteriorised spleen is then tucked into the skin pocket and the skin incision closed using non-absorbable sutures.

Prophylactic: Injectable antibiotic (pen/strep) is administered.

Post-operative care: Skin sutures are removed after at least 10 days. The animal should not be put through a race or crush after the operation to prevent damage to its vulnerable spleen.

c) Needle biopsy after exteriorisation

The skin overlying the exteriorised piece of spleen is clipped and swabbed with 70% ethanol. A 14 gauge needle with syringe containing some ASLEF's solution attached, is pushed through the skin and into the tissue of the underlying spleen. The plunger of the syringe is withdrawn until the required amount of spleen cells are drawn into the syringe. The needle is removed and pressure applied to the puncture wound until bleeding has stopped.

d) Splenectomy

Species: Cattle

Special conditions: This procedure is limited to calves less than 12 months old. In older cattle the spleen is too closely adhered to the rumen.

Site: A laparotomy in the left paralumbar fossa.

Restraint: The animal to be held by the neck in a crush with easy access to its flank.

Preparation: The animal must be starved for at least 24 hours and water withheld for 12 hours prior to surgery. Xylazine is administered at dose of 0.05 mg/kg (sedation but animal remains standing). Local anaesthetic is infiltrated around the site to be incised. The left paralumbar fossa is clipped and scrubbed with a dilute savlon solution. The site then swabbed with 70% ethanol.

Equipment: A sterile surgical kit plus a pair of long clamping forceps (spleen pean).

Procedure: Strict aseptic technique must be adhered to throughout the operation. A vertical incision is made starting 5 cm below the transverse process of the 4th lumbar vertebra extending for around 15 cm. Skin, musculature and peritoneum must be incised. Care must be taken when incising peritoneum as the rumen is in very close proximity underneath. The surgeon’s hand is passed anteriorly over the rumen until the spleen is felt. Connective tissue between the spleen and the diaphragm is separated. The splenic artery and vein are located in the hilus, in the dorsal third of the visceral surface. The arterial pulse can be felt quite easily. The artery and vein are isolated and clamped with the long forceps. Separation of the spleen from the rumen is achieved by blunt dissection. The splenic artery and vein are

cut on the splenic side of the forceps and the spleen then removed from the abdomen. The splenic artery and vein are ligated prior to removal of the forceps.

The abdominal musculature is sutured in one layer with simple interrupted catgut sutures; the skin is sutured with non-absorbable sutures.

Prophylactic: Injectable antibiotic (pen/strep) is administered.

Post-operative care: Skin sutures are removed after at least 10 days.

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11. *Theileria*

a) Infection by needle

i) Infection/treatment for *Theileria*

Species: Cattle

Background: The infection and treatment method of immunisation is based on the findings that tetracyclines can reduce the severity of East Coast Fever (ECF) in cattle when given at the time of *Theileria parva* infection or soon afterwards. The current method involves ground up tick stabilate infection of cattle with simultaneous oxytetracycline (OTC) treatment. The stabilate dose is normally determined empirically and is one which should induce severe infections in all susceptible untreated cattle. Treatement with OTC is given as a single dose of 20 mg/kg of long acting preparations or two doses (20 mg/kg each dose) given at the time of infection and repeated after four days with virulent parasite stocks. Very occasionally a four day course of short acting OTC is used at 10 mg/kg.

Immunisation procedure

a) Cattle are weighed before use to determine drug volume required.

b) Stabilate is innoculated, usually as a 1.0 ml. volume, subcutaneously below and in front of a parotid lymph gland, using a 1 ml. syringe and a 21 gauge × 3 cm. needle. The site is swabbed with 70 % alcohol prior to the innoculation.

c) At the same time the required volume of OTC is injected into the rump or thigh by deep intramuscular injection using and appropriate size of syringe and a 16 × 3 cm. needle. When more than 20 ml of the drug is required, the drug is equally divided and place into more than one site. For file applications or where large numbers of cattle are being used the OTC must be given first.

Following immunisation, cattle are examined daily and rectal temperatures taken and recorded. If fever (39.5 deg C) is detected lymph node biopsies are taken. If cattle developed severe ECF they are treated with an anti-theilerial drug such as parvaquone, buparvquone or halofuginone. Blood samples are normally taken at weekly intervals to determine serological responses to immunisation.

ii) Infection with *Theileria*

Species: Cattle

Infection procedure: Stabilate is innoculated, usually as a 1.0 ml. volume, subcutaneously below and in front of a parotid lymph gland, using a 1 ml. syringe and a 21 gauge × 3 cm. needle. The site is swabbed with 70 % alcohol prior to the innoculation.

Following immunisation, cattle are examined daily and rectal temperatures taken and recorded. If fever (39.5 deg C) is detected lymph node biopsies are taken. If cattle developed severe ECF they are treated with an anti-theilerial drug such as parvaquone, buparvquone or halofuginone. Blood samples are normally taken at weekly intervals to determine serological responses to immunisation.

iii) Inoculation of *Theileria parva* schizont-infected cells

Species: Cattle

Background: Theileria parva schizont-infected cell lines can be used to infect cattle. Normally 10^3–10^5 infected cells produce infections in autologous cattle and 10^8–10^9 in the heterologous situation.

Procedure

1. Cells from a suitable volume of culture are pelleted by centrifugation at 200g for 10 min.
2. Cells are re-suspended in a medium with a low serum content e.g. L15–5% FBS. The volume of medium used depends on the cell numbers and is usually between 5–20 ml.
3. Cattle are inoculated subcutaneously using a 16-gauge × 11/2 in needle at 1 to 4 sites i.e. over right/left parotid and prescapular lymph glands. The number of sites used depends upon the cell numbers inoculated. For 10^3 cells, one site would normally be used, but for 10^9 cells, all 4 sites are commonly used. Following inoculation, cattle are monitored daily for infection.

b) Infection of/by vector

I) Feeding clean ticks on infected cattle—feeding infected ticks on clean cattle

Species: Cattle

Animal selection: Cattle of 6–12 months of age are selected for these experiments which have been shown to be free of *T. parva* antibodies. The cattle used are usually Boran of either sex which have been brought to ILRI from Kapiti at a few days of age. These cattle are allocated to the Tick Unit when they are weaned at ILRI.

Preparation: All cattle that are to be moved to the Tick Unit are maintained off spray for at least 14 days prior to being moved to the unit. Infected cattle are usually used in groups of 4 and are infected with *T. parva* tick stabilate (see Infection with *Theileria)* in the New Facility then moved into the Tick Unit 10 days after infection. The cattle are provided with water and ranch cubes *ad lib* and are restrained by halters. The infected cattle are monitored for the development of infection by lymph node biopsies and peripheral blood smears and haematology is determined from EDTA vacutainer samples (3 mls) taken from the jugular vein 3 times weekly. Rectal temperature is taken daily. To prolong infection, cattle are treated with oxytetracycline (10 mg/kg bwt) by intramuscular inoculation daily from day 10 to 14 after infection.

Procedure: The backs or ears of the cattle are shaved using large animal electric clippers. The cloth ear bags or cloth back patches are applied and stuck in place using a rubber glue (Pattex, Henkel Chemical, Nairobi). Clean nymphae are applied on day 12,13 and 14 after infection (12,000 nymphae) on each occasion and the replete nymphae are collected on day 16, 17, 18, 19 and 20 after infection of the cattle. On day 20 after infection a decision is taken as to whether the cattle are either euthanized, if they are clinically distressed, or if not, they are treated with parvaquone or buparvaquone and returned to the farm after they recover. Animals with severe ECF prior to collection of replete nymphae will be euthanased or treated.

ii) Feeding infected ticks on rabbits

This procedure is undertaken to assess the *Theileria* infections in the salivary glands of ticks fed for four days or to obtain *Theileria* sporozoites for research purposes or stabilate preparation.

Procedure: Is similar to feeding clean ticks on rabbits. These procedures are carried out in the infected rabbit room. The feeding of infected ticks is done routinely for adult ticks and occasionally for nymphae which are fed for 3 days. For assessment of infection and harvesting sporozoites about 200 adult ticks are applied to each ear. For stabilate preparation, 400 ticks are applied to each ear. On the fourth day after attachment, the ticks are removed carefully from the ears of the rabbit. The rabbits are then either euthanized or returned to the Small Animal Unit depending on the state of their ears.

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12. Trypanosomes

a) Infection by needle

i) Cattle and goats

Bloodstream-form trypanosomes are inoculated into cattle or goats intramuscularly, intravenously or subcutaneously, and occasionally intradermally. Metacyclic-form trypanosomes are usually administered either intravenously or subcutaneously.

Intradermal inoculation: A 1–1.5 cm needle of gauge 25/26 is used, and inserted into the dermis in the flank region. An inoculation volume of approximately 0.1 ml is usually used. Prior to insertion of the needle the hair at the inoculation site is removed, first with clippers, and then with a scalpel blade or razor.

Intramuscular inoculation: A 3 cm 20–23 guage needle is used. Three capillaries of cryopreserved stabilate are thawed and diluted in 3 ml of the phosphate-buffered saline glucose (PSG, pH 8.0) is used, and is administered slowly deep into the muscle of the thigh or neck.

Intravenous inoculation: A 2–3 cm. needle of gauge 18–22 is inserted into the jugular vein. For inoculation into either the ear vein or tail vein a 3cm 20–23 gauge needle is used. Inoculation volumes are usually less than 5.0 ml. and must be administered slowly.

Subcutaneous inoculation: A 2–3 cm. needle of gauge 19–22 is inserted into whatever subcutaneous site is suitable for the research purpose. The flank or lateral neck areas are used. Inoculation volumes are less than 2.0 ml.

ii) Rabbits

Intraperitoneal inoculation: The animal is restrained with one hand holding the scruff at the back of the neck, the lower body is supported and the animal is tilted head downwards so that the abdominal contents fall away from the lower abdominal wall. The needle is inserted in the right inguinal region in a cranial direction first through the skin and then through the musculature into the abdominal cavity. The plunger is then sucked back to ensure no vessels have been penetrated. The procedure is the same as for rodents for intraperitoneal inoculation with the animal being restrained by one person and inoculated by a second. The maximum volume of inoculum is 5.0 ml.

Intravenous inoculation?

Site: Marginal ear vein

Procedure: The rabbit is restrained either in a restraining box or wrapped in a towel or lab. coat. The hair on the ear is then plucked in the region to be injected. The ear is then be massaged to dilate the vessel. Site is swabbed with 70% alcohol, the vessel occluded below the injection site (nearer the rabbit's head), this will cause dilation of the vein. The needle (maximum size 23 × 2.5 cm.) is inserted into the vein almost parallel to the vessel surface. The plunder is withdrawn until a small volume of blood is seen in the syringe. The occluding pressure is then released. The material is then injected slowly into the vein. If an increase in resistance is felt on the plunger, observe to ensure that the needle is still well positioned within the vein. On completion the site is gently squeezed and the needle withdrawn.

iii) Rodents

Bloodstream-form and metacyclic-form trypanosomes are the normal life cycle stages administered to rodents via the intraperitoneal route. However, for some purposes, particularly cloning, they may be inoculated intravenously.

Intraperitoneal inoculation: A 2–3 cm. 23–25 gauge needle is used. The animal is restrained in one's hand with the head lower in order for the abdominal contents to fall away from the lower abdominal wall. The needle is inserted in the right inguinal region in a cranial direction first through the skin and then through the musculature into the abdominal cavity. The plunger is then sucked back to ensure no vessels have been penetrated. The maximum inoculation volumes of 1.0 ml and 2.0 ml for the mouse and rat respectively may be used.

Intravenous inoculation: The rodent is first placed under a 60 watt lamp under constant observation until the tail veins are observed to be prominent. Thereafter the rodent is placed in the barrel of a 60 ml syringe for mice or a restraint device for rats and the tail secured in a rubber bung, containing a groove that is placed over the open end of the syringe barrel. A 1–2 cm. 26/27-gauge needle is then inserted into any one of the tail veins. The maximum volume for inoculation via this route is 0.25 ml. Animals should not be sedated for this procedure, as this would involve an injection with a second needle.

b) Infection of/by vector

i) Feeding non-infected tsetse flies on infected cattle and goats

Cattle or goats with *Trypanosoma vivax, T. congolense* or *T. brucei brucei* are used to infect tsetse with these pathogenic trypanosome species by feeding on the infected hosts.

Species: Cattle or goats

Special conditions: Cattle and goats must be off spray for at least two weeks prior to feeding of the flies. At least 40 days notice must be given to the Head of the Tsetse Vector Unit that flies are required to be fed on experimental animals.

Restraint: Cattle are held in a crush and goats in a head restraint.

Procedure: The flanks are shaved with electric clippers and then sterilised with 70% ethanol on absorbent cotton wool. Cages of tsetse flies (Geigy cages) are strapped onto the flanks of the animal, and the tsetse flies are allowed to feed for 10 minutes, after which other tsetse in cages are similarly fed on the same animal. Tsetse are fed on the same infected host every day, except weekends, for 25 days in the case of *T. vivax* and *T. congolense* and 30 days in the case of *T. b. brucei* infection.

ii) Feeding infected tsetse flies on cattle or goats

As for procedure of feeding non-infected tsetse flies above, except that individual tsetse infected with *T. vivax, T. congolense or T. b. brucei,* in a 8.5 × 2.5 cm tube with a cork at one end and netting at the feeding end is placed on predetermined site on the flank of the animal and allowed to engorge. Between 1 and 20 tsetse are thus used to infect or challenge each animal. The number of infected tsetse to be used and the site on the animal the tsetse are allowed to feed can alter according to the experimental protocol.

iii) Feeding infected tsetse flies on rodents

To determine the transmission rate or frequency of infection by infected tsetse.

Procedure: Each animal is held gently by the skin of its neck and dorsal body. An infected tsetse in a 8.5 × 2.5 cm. tube with a cork at one end and netting at the feeding end, is placed on its abdomen, and thus allowed to engorge.

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13. Vector Feeding (parasite not present)

a) Ticks

All tick feeding on animals is carried out in specific and designated areas of the ILRI Tick Unit which are quarantine areas. Ticks are routinely fed on animals for maintenance of laboratory colonies of ticks, infection of ticks with pathogens and the transmission of pathogens to animals. The ticks are either fed on the ears or backs of animals.

i) Feeding ticks on cattle

This procedure is used to amplify stocks of tick species required for experiments by feeding adult ticks on cattle free of tick-borne disease. The engorged female ticks are collected on repletion and allowed to lay eggs.

Cattle are selected at 6–10 months of age and brought in from the ILRI farm where they had been kept off acaricide spraying for two weeks. The cattle are allocated to a colour-coded security pen. Cattle are provided with water and ranch cubes *ad lib*.

Restraint: The cattle are restrained by a head halter.

Procedure: The backs or ears of the cattle are shaved using large animal electrical clippers. The cloth ear bags or cloth back patches are applied and stuck in place using a rubber glue (Pattex, Henkel Chemical, Nairobi). About 1400 adult ticks are applied to each back patch and up to six back patches can be applied on individual animals. Most tick species take up to one week to become replete. The exceptions are *Boophilus* species. These are one-host ticks, which take at least three weeks to complete their life cycle and produce replete females. After the ticks have become replete the cloth patches are removed and the cattle are sprayed. These cattle are returned to the ILRI farm after use or euthanised. Post-manipulative care is extremely important.

ii) Feeding ticks on rabbits

The objectives are to obtain replete instars of different tick species and stocks for routine laboratory maintenance of the tick colonies. About 15 different species or stocks are maintained for research purposes.

Procedure: The rabbits are obtained from the Small Animal Unit and placed in cages in the clean rabbit room. The ears of the rabbits are shaved using small animal electrical clippers and cloth ear bags are attached to the ears using zinc oxide plaster. Approximately three thousand larvae, or one thousand nymphae or 200 adult ticks are applied to each ear. The back legs of rabbits are taped together loosely to prevent the animals from scratching the ear bags. The rabbits are provided with water and rabbit pellets *ad lib* and the cages cleaned daily. The replete ticks are collected within a week after application and, depending on the condition of the ears at the end of feeding, the rabbits are either returned to the Small Animal Unit or euthanized.

b) Tsetse flies

i) Feeding tsetse flies on cattle or goats

Special conditions: Animals must be off spray fourteen days prior to use.

Restraint: Each animal is restrained in a head restraint.

Procedure: The flanks are shaved with electric clippers, and then sterilized with 70% ethanol on sterile surgical cotton wool. Tsetse held usually in Geigy cages are placed on the shaved area of the animal, secured by restraining straps and covered with a black cloth. Female tsetse are allowed to feed for 10 minutes whilst males are allowed to feed for 5 minutes daily. Tsetse flies in the production colonies are fed every day except weekends for 90 days.

i) Feeding tsetse flies on rabbits

Restraint: Rabbits are placed singly in a close-fitting box provided with two ear-rests. A soft pad, provided with elastic straps, is placed on the rest, the ear is laid on the pad, the cage of tsetse flies placed on top of the ear and the whole secured with the straps. The wooden end of the box is provided with a 7 cm thick foam rubber and the top of the box has a 6 cm wide rubber strap in order to reduce the risk of a compression fracture of the spine if the rabbit kicks backwards or jumps upwards during feeding.

Procedure: The ears are swabbed with 70% ethanol and then dried with tissues. 40 tsetse flies per rabbit are allowed to feed for 10 minutes every day, except Sundays. A group of rabbits (upto 40 rabbits per feeding session) are used for tsetse feeding for 3 consecutive days after which another group of rabbits is introduced for tsetse feeds, so that each group of rabbits rests from tsetse feeds for a period of 5 days. In a tsetse feeding day, each rabbit is exposed up to a maximum of 600 tsetse flies.

The rabbitry has good ventilation, and all the rabbit cages trays are cleaned twice each week. The rabbits in the Tsetse Unit are fed on a commercial coccidiostat-free rabbit pellets and have ad libitum access to water.

14. Useful Data

Biological

	Mouse	Rat	Rabbit
Adult weight (g)	20–40	450–520	900–6000
Diploid #	40	42	44
Food Intake	15g/100g bdy wt.	10g/100g bdy wt.	5g/100g bdy
Water intake	15ml/100g bdy wt.	10ml/100g bdy wt.	10ml/100g/b
Lifespan (years)	1.5–3	3–4	6–12
Rectal Temp. deg C	38–39	36–40	38.5–40
Heart rate/min	310 –840	250–450	130–325
Blood Pressure	systole mmHg	133–160	84–134
diastole mmHg	90–110	60	57–65
Blood volume (ml/kg)	60–75	54–70	57–65
Respiratory rate/min.	60–220	70–115	30–60
Tidal volume (ml)	0.18	0.6–2	4–6
Haematological data			
RBC(× 10^6/mm^3)	7–12.5	7–10	4–7
PCV (%)	39–49	36–48	36–48
Hb(g/100ml)	10.2–16.6	11–18	10–15.5
3 3			
WBC(× 10^3/ mm^3)	6–15	6–17	9–11
Neutrophils(%)	10–40	9–34	20–75*
Lymphocytes(%)	55–95	65–85	30–85
Eosinophils(%)	0–4	0–6	0–4
Monocytes(%)	0.1–3.5	0–5	1–4
Basophils(%)	0–0.3	0–1.5	2–7
Platelets (× 103/mm3)	160–410	500–1300	250–270

* Neutrophils often resemble eosinophils due to cytoplasmic granules

Biochemical data

Serum Protein (mg/100ml)	3.5–7.2	5.6–7.6	5.4–7.5
Albumin (g/100ml)	2.5.–4.8	3.8.–4.8	2.7–4.6
Globulin (g/100ml)	0.6	1.8–3	1.5–2.8
Glucose (mg/100ml)	62–175	50–135	75–150
Blood urea nitrogen (mg/100ml)	12–28	15–21	17–23.5
Creatinine (mg/100ml)	0.3–1	0.2–0.8	0.8–1.8
Total bilirubin (mg/100ml)	0.1–0.9	0.2–0.55	0.25–0.74
Cholesterol (mg/100ml)	26–82	40–130	35–53

Cattle

Biological Data

Adult weight (KG)	Male 660–1000 Female 400–800
Food Intake (KG)	< 90kg grass
Depends on reproductive cycle	< 70 kg silage and concentrates
Water intake	*ad libitum*
Lifespan(years)	15–20
Rectal Temperature	38–39
Heart rate/min	40–100
Blood volume (ml/kg)	57–62
Respiratory rate	27–40

Haematological Data

RBC (× 106/mm3)	5–10
PCV (%)	24–46
Hb (g/100ml)	8–15
WBC (× 103/mm3)	4–12
Neutrophils (%)	6–45
Lymphocytes (%)	18–75
Eosinophils (%)	2
Monocytes (%)	1–7
Basophils (%)	Rare
Platelets (× 103/mm3)	100–800

Biochemical data

Serum protein (g/100ml)	5.3–7.5
Albumin (g/100ml)	2.1–3.6
Globulin (g/100ml)	3–5.5
Glucose (mmol/l)	2–3.2
Blood urea nitrogen (mmol/l)	2–6.6
Creatinine (μg mol/l)	44–165
Total bilirubin (μg mol/l)	0–6.5
Cholesterol (mmol/l)	1–3

Blood Volumes

Species	Blood volume ml/kg	Total blood volume normal adult (ml)	Safe volume of single bleed (ml)	Practical Diagnostic volume (ml)
Mouse	58. 5	1.0–2.4	0.1–0.2	0.1
Rat	54–70	16–33	1.6–3.3	0.3
Rabbit	57–65	58.5–585	5–50	1
Sheep	58–64	4060–4480	400–450	>1
Goats	57–90	3990–6300	400–630	>1
Cattle	60	27000–36000	2700–3600	>1

The lower figures are for females in the small animals. There are large breed/strain differences in all species, these are only guidelines.

Administration volumes for laboratory animal species

Species:	Intravenous (ml)	Intraperitoneal (ml)	Intramuscular (ml/site)	Subcutaneou (ml/site)
Mouse	0.2	2–3	0.05	0.5
Rat	1	5–10	0.1	1–2
Rabbit	1–10	50–100	0.5–1	1–5

Suggested hypodermic needle guages (adult animals)

Species:	Intravenous		Intraperitoneal		Intramuscular	Subcutaneous	Intradermal
Mouse	27–28	25 –27	27	25	27		
Rat	25–27	23–25	25	25	25		
Rabbit	23–25	21–23	23–25	21–25	21–25		
Sheep	19–21	19–21	21	19–21	21		
Goat	19–21	19–21	21	19–21	21		
Cattle	14–18	14–18	18	18–21	18		

15. Castration

Species: male cattle, from birth to 4 months.

Restraint: standing, physical restraint.

Preparation: strict aseptic technique must be adhered to throughout the operation. The scrotum is scrubbed with dilute Savlon solution.

Equipment: a sterile surgical blade.

Procedure: the scrotum is grasped, squeezing the testicle against the skin. An incision is made through the skin and the tunica vaginalis over the testicle. The testicle is pulled out and the tunica vaginalis is separated from the distal border. The testicular artery and vein are pulled so that they break as far proximal to the venous plexus as possible. The second testicle is removed in the same way. The incision wounds are sprayed with antibiotic spray. Ensure calves are on clean bedding post-operatively.

16. RUMEN FISTULATION OF BIG RUMINANTS

Preparation:

The animal is starved of food and water for 24 hours before the operation.

Anaesthesia:

Rompun 0.12ml per 50kg or 0.25ml per 100kg/body weight intramuscular is used to sedate the animal. Xylocaine 0.5% or locovetic for local infiltration anaesthesia and paravertebral nerve block.

Instruments:

The following instruments are needed:

- Wound retractors
- Scalpel
- Scissors
- Artery forceps
- Rumen forceps
- Forceps
- Suture material
- Curved triangular needles
- Swabs

Method:

The operation is carried out on the standing animal in a suitable restraining crush.

Rumen fistulate are made on the left side of the animal in the anterodorsal portion of the flank and into the dorsal sac of the rumen. In this position, leakages around the cannula are minimal.

The skin of the left flank is shaved and disinfected. A skin incision (9-13cm long) is made three finger breadths behind and parallel to the last rib, beginning 3-4cm below the transverse processes of the lumbar vertebrae (the length of the incision depends on the size of the cannula).

Wound retractors are used to separate the edges. The muscles (mm obliguus abdominalis externus and internus) are separated by blunt dissection. After, the incision is made on fascia and peritoneum. Penicillin and streptomycin powder are introduced into the abdominal cavity through the wound. Then, the dorsal sac of the rumen is located and lifted through the wound. The rumen is fixed with two pairs of rumen forceps, one on the upper part of the rumen wall and the second on the lower part. Then the skin, peritoneum and rumen wall are stitched together around the edges of the wound with interrupted U-sutures of silk. Care must be taken not to pierce the wall of the rumen (stitch not deeper than the

rumen muscularia). Then, that part of the rumen lying between the two rumen forceps is now opened. At about the middle an incision is made with a scalpel and enlarged upwards and downwards with scissors and the wall of the opened rumen is folded back to the skin. Finally, the internal flange of the cannula is folded to enable the cannula to be inserted into the fistula.

To prevent post-operative infection, the animal is given broad spectrum antibiotics intramuscular for 7 days, the wound is dressed daily with aerosol antibiotics. The wound usually heals in 2-3 weeks.

17a. INFECTION OF SHEEP WITH INFECTIVE NEMATODE LARVAE

Restraint: Manual

a. Cleaning the sheep

If the infection is for maintaining a larval culture, ensure that the sheep to be used are free of prior infection by treating them at least 20 days before the intended artificial infection. Give two recommended doses of a recommended anthelmintic at a 12-24 hour interval and maintain the sheep in worm-free conditions by either

- Housing them on a slatted floor or
- Cleaning their pens at least twice a week.

In addition, the feeding troughs should be constructed such that the feeds in them are not liable to be contamination with faeces. Take faecal samples 15-20 days after the second treatment and examine for nematode eggs to ensure that the animals are worm-free (clean).

a. Infection

Estimate the number of larvae that are present per ml of a larval suspension in tap water. Aliquot the suspension in volumes that contain the required number of larvae for infection and transfer into 15 ml test tubes for oral infection. Place the tube over the tongue and direct the larval suspension down the throat. Rinse the tube 3 times with tap water and empty the contents down the throat.

17b. COLLECTION OF FAECAL SAMPLES

Spp: Sheep, Cattle

Restraint: Manual

Procedure: Faecal samples are preferably collected from the rectum and examined fresh. Insert the forefinger of your gloved hand into the rectum and push out the faeces. The glove can be turned inside out to act as the receptacle. Alternatively, the faeces are transferred into a polythene bag of about 5 cm x 8 cm or into any other suitable container. Label the sample with the animal's tag number.

NB. About 5 g of faeces should be collected. If examination is not possible on the day of sampling, the samples must be stored in a refrigerator since nematode eggs embryonate rapidly.

17c. COLLECTION OF FAECES FROM 'CULTURE' SHEEP

'Culture' sheep must be males and they should be big enough to be fitted with harnesses. The sheep will have been treated to render them worm-free as described in section a above.

The harness is fitted and a bag for faecal collection suspended on the harness at the rear of the animal. The bag is left in place overnight; it is removed in the morning and its contents incubated to yield larvae.

18a. INFECTION OF RODENTS WITH INFECTIVE NEMATODE LARVAE

(This protocol is specifically for *Heligmosomoides polygyrus* in mice)

Restraint:

Mice are infected normally without the use of anaesthetic. They should be held firmly by the loose skin on the neck, and if necessary the tail pinned to the palm of the hand by the 3rd and 4th finger (Consult a standard manual on handling laboratory rodents, e.g. UFAW Handbook). Gloves may be worn because rodent urine and hair can cause allergies, but for most personnel this will not be required. If the handler is uncomfortable with this procedure, temporary anaesthetic may be used.

The animals:

If the infection is for routine maintenance of the parasite, about 10-12 mice should be used. These should be worm free. Mice may carry the pinworms *Aspiculuris tetraptera* and *Syphacia obvelata* and rats *Syphacia muris.* Both may also be infected with the cestode (tapeworm) *Rodentolepis* (*Hymenolepis*) *nana*. The former species can be eradicated either by individual dosing with piperazine or by providing piperazine in the drinking water for at least one week, and then repeating the procedure after a week of rest. In both cases the animals will need to be rested (provided with fresh drinking water) for at least one week before infection, and after treatment their bedding should be changed frequently (daily of every 2 days) to ensure that all vacated worms are eliminated and that their eggs do not lead to fresh infections. *R. nana* is more difficult to treat but the worms can be removed by dosing with niclosamide.

For routine passage a strain of mouse capable of supporting a long lasting infection should be used to avoid frequent infections. For this CBA, C57BL10 and C3H (poor responders) mice are ideal. All these strain carry an infection that will last up to 10 months in duration. Avoid using SWR, SJL, NIH and BALB/c mice, since these strains lose worms within 6-7 and 10-15 weeks respectively. If outbred stock such as Swiss mice are available, these can be used but more frequent passage is advised.

Fresh batches of mice should be infected at 2 (for outbred strains) -3 (for CBAs and other poor responders) month intervals, and at any one time there should be 3 batches of mice carrying infections of varying age (e.g. freshly infected, 2 month and 4 month infections). When a fourth batch is infected, the oldest batch should be disposed of.

The poor responder strains should be infected with 150-200 larvae. Larger doses may result in mortality. Outbred strains may be given more but 500L3s should not be exceeded for any strain if long-term survival is required. It is in fact counterproductive to employ heavier doses because density-dependent suppression of fecundity has been reported for *H. polygyrus* in mice.

Infection

Third stage infective larvae should be prepared in distilled water in a conical flask. For the purpose of estimating the larval concentration, the larvae can be held in even suspension by employing a magnetic stirrer. This approach should always be used when many mice require to be infected and particularly when the infections are for experimental purposes and accurate delivery of larvae is important. A short cut (when just a few mice require to be infected and when the intensity of infection is not critical) is simply to invert a universal containing the larvae several times and to take an aliquot rapidly after removal of the lid.

|Larval counts should always be carried out on larvae removed immediately from the fridge where they can be left at about 4C for many months. The larvae even survive short term freezing, but this is not recommended as routine. Place the flask on a magnetic stirrer, add the magnetic flea and stir at a slow rate, sufficient to bring all larvae into suspension but not fast enough to damage them. Take 0.1ml aliquot from the middle and empty this into a Petri dish in several small drops. Count the number of active larvae in each. Repeat this for at least 5 counts and then estimate the average in 0.2ml. From the total volume of fluid and the average concentration in your samples, calculate the additional volume,

which is required to dilute to the desired concentration and add water, or, if the initial concentration is too dilute, allow the larvae to stand for about 15min to allow sedimentation and then remove the required volume.

You should always aim to administer the required dose in 0.2ml of fluid but this is not critical and 0.05ml (much more difficult to obtain consistent worm burdens) and 0.3ml (approaching stomach limit and risking regurgitation) can also be used.

Mice should be infected orally (gavage) using a blunted wide bore needle on a 1ml syringe or a specially adapted needle with a ball end. Each batch of larvae should be removed separately. Do not take up 2 or more doses at one time, because by the time you have infected the first mouse larvae will have sedimented out and the first mouse will have received double the dose and the second very few larvae.

Monitoring the mice during infection
Infected mice should be inspected daily, especially in the first 3 weeks following infection, to ensure that none are suffering distress from excessively heavy parasite burdens. The initial symptoms to look out for are scruffy fur, sitting alone in the cage away from other mice and lethargic movements. In heavy infections the animals gradually become emaciated (note loss of muscle on limbs and fat from the face), the face becomes more pointed and anaemia may become evident. The face, ears and limb extremities become very pale. The extreme symptoms may set in rapidly and the animal can deteriorate within several hours and die.

If caught early enough such animals can be treated with pyrantel to remove the worms, or better, should be destroyed humanely by an approved method.

18b COLLECTION OF FAECAL SAMPLES from Rodents

Spp: Rodents, esp mice

Restraint: none

For routine culture/passage

Procedure: Mice should be placed into a cage with a grid base, and this should be arranged over a tray containing moist (not water logged) tissue paper. The mice should be kept in these cages overnight with food and water provided *ad libitum*. On the following day, scoop up as much of the faeces as possible into a beaker avoiding contamination with food, as much as possible. Add about half the volume of charcoal grains (not fine powder), mix, add water sufficient to generate a slurry. This should not be so fluid as to run loosely over a glass surface.

Spread the slurry thinly (**if the slurry is left too thick the larval recovery will be poor**) on filter paper in the bottom of a standard Petri dish. The filter paper should be smaller in diameter, leaving about 0.5-1cm margin of open glass all the way around the circumference of the paper. Spread the slurry out from the middle of the filter paper but stay clear of the edge and again leave a 0.5 –1cm clear margin of paper. Wait for the moisture from the slurry to spread and if any parts of the filter paper are still dry, add sufficient water to moisten the filter paper. Do not add excessive water - the paper must be moist but not water logged. Petri dishes should now be stacked in a plastic airtight box with a base lined by moist tissue paper. These boxes can be kept at 20-24C, but not higher. Under colder ambient temperature larvae will take longer to develop. The larvae will take 6-7 days, but should be inspected daily to ensure that the filter paper has not dried out.

Ideally the Petri dishes should be harvested after 10-14 days, but this is not critical and if larvae are needed desperately any time after day 7 will do although the longer the plates are left after week two, the less infective the larvae are likely to be. The larvae of *H .polygyrus* adopt a type of behaviour called nictation when ready to infect hosts. They first migrate out of the filter paper and when on the glass anchor their tails and stand vertically. If there has been no faecal contamination of the glass, they can be harvested by simply holding the Petri dish at an angle and pipetting some distilled water, about 0.5-1ml (not critical) with a pipette against the walls of the Petri dish. Revolve the dish to mop up larvae

all the way around the clean glass rim and pipette up and transfer to a conical flask. Take care to avoid contamination with faeces, so keep well away from faecal slurry and remove any larger accumulations that may detach and fall into the fluid as you rotate the Petri dish. This procedure should be repeated several times until all larvae on the glass have been mopped up. The filter paper can now be thrown away or left for another day, when more larvae will have migrated out of the faecal slurry and the procedure can be repeated.

For accurate determination of faecal egg concentration (EPGs)

Procedure: Egg counts are best estimated on fresh faeces. Mice should be transferred individually from their cages into clean plastic cages early in the morning. No food should be provided but water can be made available. After 2-3 hours, when a number of faecal pellets have been dropped they can be returned to their cages. The faeces is then collected, weighed and EPGs estimated by a McMaster or other appropriate method. Ideally a gram of faeces will be collected from each mouse but this is unlikely. EPGs can be calculated on as little as 0.1gm, but aim to collect at least 0.25gm/mouse.

18c RECOVERY OF WORMS

Spp: Rodents, esp mice

L4 stage

The infective *polygyrus* exsheath in the stomach, pass into the duodenum and penetrate through the mucosa into the submucosa. They then migrate outwards to end up lying just beneath the serosa in the *muscularis externa*. Here they develop for about 7-8 days. The first moult (L3 to L4) takes place 2-3 days after infection and the second 7-9 days after infection. The following procedures will describe recovery of worms for accurate worm counts and quantitative recovery for antigen preparation.

Quantitative but not complete recovery of L4 larvae: This procedure should not be used for accurate worm counts. To collect 6day old L4s the mice should be infected with 1000 L3s and killed on day 6 after infection. Mice should be killed by an approved method (e.g. over-exposure to carbon dioxide or by cervical dislocation [if blood is not required]). Open the peritoneal cavity, and remove the small intestine. Separate the small intestine from the stomach and caecum and divide roughly in half. The second half will contain most of the bulk gut contents and very few worms and can be thrown away. Now open the anterior half of the small intestine longitudinally with round tipped small scissors. Using forceps shake hold in a beaker of Hanks' saline and shake out all the gut contents. Now spread on a clean bench surface with the serosal side down and the mucosa upper most. Using a clean glass slide, scrape the mucosa gently. Now place both the scrapped tissues and the scrapings onto a gauze (fine netting), suspend in a 50ml beaker of Hanks' saline and incubate at 37C for one hour. After this period, transfer the gauze to a second beaker for another 1h period of incubation. Pour the contents of the first beaker into a large boiling tube held in a bucket of ice. After about 10mins remove the supernatant since the worms will have settled on the bottom. Pool the contents of several beakers in the way. When the second hour has elapsed repeat the procedure and if substantial worm burdens still persist repeat again for a third hour. When all the incubations have been completed pool all the worms in one beaker, wash 10 times in ice cold Hanks' by repeated sedimentation and removal of the supernatant. If there is substantial contamination with host cells from the mucosa observe from the side of the boi.ling tube as sedimentation occurs and remove supernantant with cells and no worms from the top as the worms settle. Ten repeats of this should result in virtually pure worms. If sterile worms are required the worms can be washed 10 more times in ice cold PBS, the final volume reduced to a minimum and the worms frozen for use in antigen preparations.

Accurate assessment of L4 worm burdens: The assessment of L4 larval burdens is not easy and even with this technique is not totally accurate. Do not expect to be able to count every worm. The larvae reside in the *muscularis mucosa* and are difficult to see. Not all migrate out of the tissues and they are difficult to detect in inflamed intestines, as for example when immune mice are challenged with larvae.

Recover the small intestine as described above, but do not cut in half. Open longitudinally as before and wash out gut contents. Now cut into small segments from the stomach towards the posterior end. These

segments should be just shorter than a standard glass slide. Spread the first segment out so that that the serosa faces downwards onto the slide. Now place another slide on top, squash and keep the two slides together with a strong rubber band. Now examine under a low power of a microscope, with the serosal side uppermost. You will need to scan carefully so as not to miss any section of the gut. Work your way through the entire small intestine in this way and add up the worms observed in each segment to give a total L4 worm burden.

Adult worms

Accurate assessment of adult worm burdens: For recovery of adult worms mice should not be infected with more than 500 larvae at any one time. If repeated infections are to be given at weekly intervals the sum total of larvae administered over several weeks may exceed 500.

Adult worms live in the intestinal lumen, usually wrapped around villi. Often they accumulate in knots that may contain over a hundred worms (depending on dose of larvae administered). Recover the small intestine as described above, place it on a gauze (netting) that has been spread out in a Petri dish. Now cut open longitudinally, and allow all cut contents, worms and tissue to remain on the gauze. Now suspend in a 50ml beaker containing about 30ml of warm Hanks' saline. Incubate for a minimum of 4 h. If killing a large number of mice, time the 4 h incubation period from the last mouse killed. After 4 h, carefully remove the gauzes but grasping the corners so as to ensure that none of the material on the gauze falls into the beaker. Throw the tissues away, but place the gauze on a Petri dish and quickly scan under a low power to find any worms that have got caught in the meshes and not made their way through the material. Count these, or remove them, as required. In the meantime the beakers should contain large knots of worms if the mice were infected. Now increase the temperature of the water bath to 42C. When the temperature reaches 42 increase further 2 degrees at a time at 5 min intervals until the temperature exceeds 50C. This procedure causes most of the worms (but unfortunately not all) to become dissociated from the knots and this in turn facilitates easier counting.

Now allow the beakers to stand on a bench for a few minutes and remove some of the supernatant fluid with a Pasteur pipette. Check that this does not contain worms and throw away. The remaining fluid containing the worms can be poured into a standard universal (20-25ml), but remember to resuspend all the worms by stirring before pouring into the universal. Wash any remaining worms out of the beaker and ensure that none have been left by a quick examination under low power of the microscope.

If the worms are to be counted quickly (on the same day or next day) the tubes can be kept in a fridge. If they are to be stored for longer, add a pipette full (about 1-2ml) of a mixture of formalin/ethanol (50:50 volume of 40% formaldehyde solution and 100% ethanol). To count worms pour contents of tube into a Petri dish and under an appropriate magnification of a dissecting microscope, scan the dish removing worms individually and recording numbers of worms as you do so. Take care to wash out all the contents of the tube and not to miss any worms. Remaining tangles of worms will have to be carefully disentangled by gently pulling the worms apart using fine forceps. Remember to add to the total worm burden recorded here any worms which had been noted earlier on the gauze. If required, the sexes can be counted separately and smaller worms can be classified as stunted or as larvae depending on morphology.

19. SURVIVAL CRITERIA

(This protocol is specifically for mice challenged with *Trypanosma congolense* and replaces death as an endpoint)

Justification of Survival Experiments:

In earlier studies, it was shown by ILRI's Scientists and others that different mouse strains respond differently to the infection with the livestock and human pathogen Trypanosoma. The variations of the mouse response were measured as the mean survival time (MST) following trypanosome challenge. In the course of the study of *T. congolense* infection, we have tried alternative phenotypic measures, but unfortunately, these measures, such as gain/loss of body weight and PCV did not show any direct association with resistance or susceptibility of the mouse strains to the infection. So far, the mean survival time of the mouse following trypanosome challenge is the most accurate measure to show variations in the susceptibility.

However, it has been observed that most mice become sick and weak before they finally die, and it should be possible to reduce suffering of these mice, without interfering too much with the "time to death" measurement. Indeed, in preliminary experiments we monitored some criteria some of which correlated very highly with death. The suggested procedure below would make it possible to estimate time to death without having to let the animals die.

In experiments 2001.26 and 98.009 we followed 212 mice. 100 % of the mice in category 4 died within 12 hours.

Procedures:

All mice to be checked daily, in the morning. Mice rated 3 should be checked at least a second time by two people in the late afternoon. If there are concerns, mice to be checked more frequently.

Records to be maintained of condition of mice within each cage on each check using the form below that can be pasted and printed in EXCEL. Mice that have attained category 3 or 4 status to have their numbers recorded.

Condition of mice to be scored according to the following criteria.

Condition of mice	**Category**
Mice appear normal	1
Mice have abnormal posture, hair appears starey.	2
Mice as above, plus unwillingness to move within cage, change of respiration	3
Mice as above , plus will not move when placed on palm of hand and given gently prod. Mice feet feel cold to touch. Animal appears very weak.	4

At stage 4 mice to be humanely destroyed.

Example of cage record checks.

Animal's Health Status

Cage #	12/02/02		13/02/02		14/02/02		15/02/02	
	AM	PM	AM	PM	AM	PM	AM	PM
51	1	1	1	1	1	2	2	2
52	2	3 #1	3 # 1,3	3 # 1,3	3 # 1,3	4 # 1 cull	4 # 3 cull	3 # 2
53	3	3 # 4	3 # 4	2	2	2	2	2
54	2	2	2	2	2	3 # 2	3 # 2	3 # 2
55	1	1	1	1	1	1	1	1

20. ENDPOINT DETERMINATION FOR ECF IN CATTLE

Responsibilities

2 designated technicicans trained in observation *– [For any 1 trial 2 technicians should take primary responsibility to ensure continuity with 2 others to cover emergencies]*

- Collect and analyse samples each morning from day 1 and record clinical signs
- Monitor animals 3 times/day from day 10 and record clinical signs – observe animals and consult with farm staff

Veterinary surgeons –

- Check animals once daily from day 10
- On-call to respond to index received by end of day
- Euthanise animals at appropriate triggers [NB The vet will take primary responsibility for decisions on euthanasia according to the procedures and according to animal welfare standards.]

Bob King (NACWO)

- Monitor animals at least once daily and more frequently as required, consult with Vet or PI where there is concern
- Euthanise on RI trigger (current ≥ 6 AND predicted ≥ 8) if vet not available and be available to dispose of all bodies!
- Follow up if thrice daily monitoring and veterinary supervision not being applied

Statisticians

- Ensuring RIs calculated by 5.00
- Alert vet if RI current ≥ 6if RI current ≥ 6 for first time AND RI predicted ≥ 8

Daily monitoring and sampling routine

0830 Animals checked by technicians and clinical parameters recorded
Temperature recorded
Samples take for determination of parasitosis

12.30 Animals checked by technicians and clinical parameters recorded

1430 (latest) All blood parameter data to be delivered to one of 2 designated statisticians. It is critical that the timing is observed – reaction indexes MUST be calculated by the end of the day from day 13.

16.30 Animals checked by technicians and clinical parameters recorded

17.00 Reaction indexes calculated – any animals with RI current ≥ 6 for first time reported to vet. As soon as RI predicted ≥ 8 as well – then the duty vet will immediately return to the farm and euthanise the animal.

Veterinary visit: The vet should visit at least once a day from day 10. Timing of the veterinary visit is not critical, but they should be on call 24 hours a day from day 10 *[it is unlikely any callouts will occur before day 13]*.

Action triggers

1) Recumbent animal Euthanise if RI current ≥ 6 AND RI predicted ≥ 8. If RI predicted ≤ 8 it will be left to the discretion of the vet

2) If the animal is coughing OR it has nasal discharge:

The records will be consulted and
IF RI current ≥ 6 AND RI predicted ≥ 8 then the animal will be euthanised
IF RI current ≥ 6 BUT RI predicted ≤ 8 the vet will be called to look at the animal again, even if he has visited already that day

3) If RI current ≥ 6 but RI predicted < 8 then the vet will be alerted and will mark the barn records. From this point, twice daily visits by the vet will be instigated with the vet paying particular attention to those animals on subsequent visits
If RI current ≥ 6 AND RI predicted ≥ 8 the vet or Bob should be called to euthanise the animal, irrespective of any clinical observations: A clear callout list will given to the statistician and those persons should be aware they are ON CALL.

4) If, in the opinion of the vet the animal has reached a stage where it needs to be euthanised – this will be left to the vet's discretion before day 13. However they will have the right to request access to daily records of the parameters.

5) Any animal that is at reaction index (RI) below 7 on day 22 can be allowed to continue under veterinary supervision. Those of RI 7 and above will be euthanised.

ECF end point record sheet

(Also available as Excel spreadsheet)

Monitoring schedule

Day	Times	Responsible	Activity
1	8.30	Technician	Sample and record clinical signs
1	Any	Vet	Record clinical signs
2	8.30	Technician	Sample and record clinical signs
2	Any	Vet	Record clinical signs
3	8.30	Technician	Sample and record clinical signs
3	Any	Vet	Record clinical signs
4	8.30	Technician	Sample and record clinical signs
4	Any	Vet	Record clinical signs
5	8.30	Technician	Sample and record clinical signs
5	Any	Vet	Record clinical signs
6	8.30	Technician	Sample and record clinical signs
6	Any	Vet	Record clinical signs
7	8.30	Technician	Sample and record clinical signs
7	Any	Vet	Record clinical signs
8	8.30	Technician	Sample and record clinical signs
8	Any	Vet	Record clinical signs
9	8.30	Technician	Sample and record clinical signs
9	Any	Vet	Record clinical signs
10	8.30	Technician	Sample and record clinical signs
10	12.3	Technician	Record clinical signs
10	16.3	Technician	Record clinical signs
10	Any	Vet	Record clinical signs
11	8.30	Technician	Sample and record clinical signs
11	12.3	Technician	Record clinical signs
11	16.3	Technician	Record clinical signs

11	Any	Vet	Record clinical signs
12	8.30	Technician	Sample and record clinical signs
12	12.3	Technician	Record clinical signs
12	16.3	Technician	Record clinical signs
12	Any	Vet	Record clinical signs
13	8.30	Technician	Sample and record clinical signs
13	12.3	Technician	Record clinical signs
13	16.3	Technician	Record clinical signs
13	Any	Vet	Record clinical signs
14	8.30	Technician	Sample and record clinical signs
14	12.3	Technician	Record clinical signs
14	16.3	Technician	Record clinical signs
14	Any	Vet	Record clinical signs
15	8.30	Technician	Sample and record clinical signs
15	12.3	Technician	Record clinical signs
15	16.3	Technician	Record clinical signs
15	Any	Vet	Record clinical signs
16	8.30	Technician	Sample and record clinical signs
16	12.3	Technician	Record clinical signs
16	16.3	Technician	Record clinical signs
16	Any	Vet	Record clinical signs
17	8.30	Technician	Sample and record clinical signs
17	12.3	Technician	Record clinical signs
17	16.3	Technician	Record clinical signs
17	Any	Vet	Record clinical signs
18	8.30	Technician	Sample and record clinical signs
18	12.3	Technician	Record clinical signs
18	16.3	Technician	Record clinical signs
18	Any	Vet	Record clinical signs

19	8.30	Technician	Sample and record clinical signs
19	12.3	Technician	Record clinical signs
19	16.3	Technician	Record clinical signs
19	Any	Vet	Record clinical signs
20	8.30	Technician	Sample and record clinical signs
20	12.3	Technician	Record clinical signs
20	16.3	Technician	Record clinical signs
20	Any	Vet	Record clinical signs
21	8.30	Technician	Sample and record clinical signs
21	12.3	Technician	Record clinical signs
21	16.3	Technician	Record clinical signs
21	Any	Vet	Record clinical signs
22	8.30	Technician	Sample and record clinical signs
22	12.3	Technician	Record clinical signs
22	16.3	Technician	Record clinical signs
22	Any	Vet	Record clinical signs
23	8.30	Technician	Sample and record clinical signs
23	12.3	Technician	Record clinical signs
23	16.3	Technician	Record clinical signs
23	Any	Vet	Record clinical signs

21. DEFINITION OF THE END POINTS FOR TRYPANOSOME INFECTIONS IN CATTLE, GOATS AND SHEEP

Criteria to be used for termination of trypanosome infections in cattle, goats and sheep.

Two criteria that should be used are:

(a) PCV values

(b) Clinical assessment

The application of these criteria to experimental trypanosome infections in livestock should be carried out as follows:

1. Infections with standard populations of *T. congolense*, *T. vivax* and *T. brucei* (i.e., populations that do not produce a haemorrhagic syndrome and that do not invade the central nervous system [CNS]) in goats, sheep and cattle with less than 50% N'Dama genes:

(a) When **PCV** values are **greater than 15%** the PCV should be determined at least **twice a week** in all animals. Similarly, all such animals should be examined at least **twice a week** by a veterinarian (preferably the PI). The PI must be involved in the process.

(b) When **PCV** values are **13-15%** the PCV should be determined at least **three times a week** in all animals. All such animals should be **examined daily** by a veterinarian (preferably the PI). The PI must be involved in the process.

(c) When **PCV** values are **less than or equal to 12%** experimental work with any such animal should be terminated as per SOPs for treatment of trypanosome infections or euthanasia.

Additional criteria for termination of experimental work (at the discretion of the duty veterinarian):

(i) recumbency for more than 24 hours

(ii) any clinical sign indicative of distress

2. Infections with standard populations of *T. congolense*, *T. vivax* and *T. brucei* in cattle carrying 50% or more N'Dama genes:

(a) When **PCV** values are **greater than 15%** the PCV should be determined at least **once a week** in all animals. All such animals should be examined at least **twice a week** by a veterinarian (preferably the PI). The PI must be involved in the process.

(b) When **PCV** values are **12-15%** the PCV should be determined at least **twice a week** in all animals. All such animals should be **examined three times a week** by a veterinarian (preferably the PI). The PI must be involved in the process.

(c) When **PCV** values are **less than 12%** experimental work with any such animal should be terminated as per SOPs for treatment of trypanosome infections or euthanasia.

Additional criteria for termination of experimental work (at the discretion of the duty veterinarian):

(i) recumbency for more than 24 hours

(ii) any clinical sign indicative of distress

3. Infections with haemorrhagic *T. vivax*:

(a) **From day 1** of infection the PCV of all animals should be determined **at least twice a week**. Similarly, all the animals should be examined at least twice a week by a veterinarian (preferably the PI).

(b) **Once an animal is detected parasitaemic** the PCV should be determined **at least three times a week**. Similarly, all such animals should be examined **at least three times a week** by a veterinarian (preferably the PI). The PI must be involved in the process.

(c) When a **PCV** value is **13-20%** this parameter should be **determined daily** in all such animals. Similarly, all animals with PCV values in this range should be **examined daily** by a veterinarian (preferably the PI). The PI must be involved in the process.

(d) When a **PCV** values is **less than or equal to 12%** experimental work with any such animal **should be terminated** as per SOPs for treatment of trypanosome infections or euthanasia.

Additional criteria for termination of experimental work (at the discretion of the duty veterinarian):

(i) recumbency for more than 24 hours

(ii) any clinical sign indicative of distress

N.B. for daily collection of small volumes of blood it may be preferable to use ear veins.

4. Trypanosome infections with CNS involvement:

(a) Once CNS signs have developed in an animal it should be **examined daily** by a veterinarian.

(b) Upon development of CNS signs an animal should remain on experiment for a **maximum of 7 days** if such signs are continually exhibited. Thereafter the animal should be euthanised (see SOPs).

(c) Any animal with CNS signs should be **euthanised immediately** (see SOPs) if:

(i) recumbent

(ii) head pressing for more than 24 hours

(iii) development of aggressive behaviour

(iv) any clinical signs indicative of distress

General point

The on-duty roster for the veterinary clinician should be available in a public folder on the LAN.